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Cephalotrichum and related synnematous fungi with notes on species from the built environment

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Abstract: A recent taxonomic revision of *Microascaceae* with an emphasis on synnematous fungi enabled re-identification of previously isolated indoor strains of *Cephalotrichum*. All available *Cephalotrichum* strains from the culture collection of the Westerdijk Institute were studied, 20 originating from the built environment. Phylogenetic relationships were inferred from DNA sequence data from the internal transcribed spacer 1 and 2 and intervening 5.8S nrDNA (ITS), and parts of β-tubulin (*tub2*) and translation elongation factor 1-α (*tef1*) genes. Additionally, herbarium material of 14 *Cephalotrichum* species described from soil in China was studied, and the taxonomy of *C. album*, not considered in recent revisions, was reevaluated. Sixteen phylogenetic species in *Cephalotrichum* are distinguished, five described as new species: *C. domesticum*, *C. lignatile*, *C. telluricum*, *C. tenuissimum* and *C. transvaalense*. Five *Cephalotrichum* species occur in the built environment: *C. domesticum*, *C. gorgonifer* (formerly known as *Trichurus spiralis*), *C. microsporum*, *C. purpureofuscum*, and *C. verrucisporum*. Based on the number of isolates, *C. gorgonifer* (nine strains) is the most common indoor species. The study of the Chinese herbarium material resulted in the acceptance of three additional *Cephalotrichum* species: *C. casteneum*, *C. ellipsoideum*, and *C. spirale*. Four species are considered nomena dubia (*C. cylindrosporum*, *C. macrosporum*, *C. ovoideum*, and *C. robustum*), five are placed in synonymy with other *Cephalotrichum* species (*C. acutisporum*, *C. inflatum*, *C. longicollum*, *C. oblongum*, *C. terricola*) and one species, *C. verrucipes*, is probably a synonym of *Penicillium clavigerum*. *Cephalotrichum columnare*, former *Doratomyces columnaris*, is transferred to *Kernia*. *Cephalotrichum album*, formerly known as *Doratomyces putredinis*, is transferred to *Acaulium* and redescribed.

Key words: *Doratomyces*, Herbarium, *Microascaceae*, *Microascales*, *Sordariomycetes*, Synnematous hyphomycetes.
Taxonomic novelties: New combination: *Acaulium album* (Costantin) Seifert & Woudenb., *Kernia columnaris* (H.J. Swart) Woudenb. & Samson; **New species:** *Cephalotrichum domesticum* Woudenb. & Seifert, *C. lignatile* Woudenb. & Seifert, *C. telluricum* Woudenb. & Seifert, *C. tenuissimum* Woudenb. & Seifert, *C. transvaalense* Woudenb. & Seifert; **Typification: Epitypification (Basionyms):** *Synpenicillium album* Costantin.

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INTRODUCTION

The genus *Cephalotrichum* is characterised by the formation of dry-spored, indeterminate synnemata and enteroblastic percurrent conidiogenesis. No sexual morph is known. It was first described by Link (1809), for two species, *C. rigescens* and *C. stemonitis*. Hughes (1958) chose *C. stemonitis* as lectotype, anchoring the modern generic concept of *Cephalotrichum*. Later, *Doratomyces* was described with *D. neesii* as its type (Corda 1829, considered a synonym of *C. stemonitis* by Hughes 1958) and later, *Stysanus* with *S. stemonitis* as its type (Corda 1837). Consideration of the type or lectotype species of these three genera, *Cephalotrichum*, *Doratomyces* and *Stysanus*, leads to the conclusion that all are typified by the fungus originally described as *Isaria stemonitis* (Abbott 2000). A later genus, *Trichurus*, with *T. cylindricus* as its type, was distinguished by the presence of sterile setae on the synnemata (Clements & Pound 1896). In the unpublished Abbott (2000) thesis on holomorph studies in the *Microascaceae*, the synonymies of the three genera *Doratomyces*, *Stysanus* and *Trichurus* under *Cephalotrichum* were proposed, conclusions followed by Seifert *et al.* (2011). These synonymies were later confirmed based on analyses of the LSU and ITS rDNA subunits (Sandoval-Denis *et al.* 2016a, b). Within *Cephalotrichum* Sandoval-Denis *et al.* (2016b) described two new species, proposed five new combinations, and designated one neotype specimen, two lectotypes and four epitypes for accepted species. Although this provides a more stable taxonomy for synnematous *Microascaceae*, the papers also highlighted a large number of taxa that could not be studied because of the absence of living cultures. Their list of uncertain or excluded species included 43 *Cephalotrichum* spp., and seven *Doratomyces* spp. These included 14 new *Cephalotrichum* species described recently from China, mostly based on morphology characters alone (Jiang & Zhang 2008, Jiang *et al.* 2011). We were fortunate to obtain herbarium material of these latter species for study, allowing us to evaluate them in the broader context of the *Cephalotrichum* taxonomy established by Sandoval-Denis *et al.* (2016b).

Most *Cephalotrichum* species occur on decaying plant material, straw, dung, wood and in soil (Domsch *et al.* 2007). They are infrequently reported from the indoor or built environment. *Cephalotrichum microsporum* (previously known as *Doratomyces microsporus*) is the species most often reported from the indoor environment (Prezant *et al.* 2008, Samson *et al.* 2010, Flannigan *et al.* 2011), where it is mentioned as occurring especially on wet cellulose-containing substrates like wood. *Cephalotrichum purpureofuscum* has also been reported from indoor air (Abbott 2000, Sandoval-Denis *et al.* 2016b) as has *C. gorgonifer* (Abbott 2000, as *C. spirale*). *Cephalotrichum* species are not regarded as human pathogens, and not known as producers of

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mycotoxins. Strains have been isolated from clinical origins, mostly human respiratory systems, but are considered passive colonisers or sample contaminants rather than active pathogens (Sandoval-Denis *et al.* 2016b). *Cephalotrichum gorgonifer*, for example, has been isolated from human clinical samples and can grow at human body temperatures (Sandoval-Denis *et al.* 2016b). However such reports are scarce and clinical data is lacking. Given the amount of time we spend indoors, it is important to understand which microorganisms are co-habitants of this environment and what their potential implications may be to human health and to the design of the built environment. For that reason, we re-evaluated the identification of newly isolated strains from house dust and other indoor substrates, and other strains from the built environment in our collections.

The aim of our project was to construct an updated phylogenetic overview of the genus, taking into account the availability of the previously unavailable species described from China, and the strains from the built environment. Cultures and specimens were also examined of an anomalous coprophilous white species, included by Morton & Smith (1963) as *Doratomyces putredinis* then later renamed as *Cephalotrichum album* (De Beer *et al.* 2013), allowing us to complete the phylogenetic analysis of the classical species of this complex that are available in pure culture.

MATERIALS AND METHODS

Isolates and herbarium specimens

Seventy-two strains belonging to the genera *Acaulium*, *Cephalotrichum*, *Graphium*, *Kernia* and *Wardomyces* were included in this study (Table 1). They were obtained from the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, the Netherlands and the working collection of the Applied and Industrial Mycology department (DTO) at the Westerdijk Institute. Strains were grown on oatmeal agar (OA) (Samson *et al.* 2010).

Portions of fourteen holotype herbarium specimens, originally accessioned in the Plant Pathology Herbarium of the Shandong Agricultural University, China (HSAUP) were recently donated to the herbarium of the Westerdijk Institute (CBS-H) and were re-examined as part of this study (Table 2). For the holotypes of these species, we have indicated the original accession numbers for holotypes from the protologue, and consider the portions deposited in CBS-H to be isotypes, for which new accession numbers are published here with the following form: "**holotype** HSAUP xxxxx → **isotype** CBS-H yyyyy." Additional isotype were listed in the protologues in HMAS; we have not examined these, but include the accession numbers as listed by the authors.

DNA sequences from six strains maintained at the UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada were obtained from GenBank (Table 1).

DNA isolation, PCR and sequencing

DNA extractions were performed using the Ultraclean® Microbial DNA Isolation Kit (MoBio laboratories, Carlsbad, CA, USA), following manufacturer's instructions. The internal transcribed spacer 1 and 2 and intervening 5.8S nrDNA (ITS), and parts of the β-tubulin (*tub2*) and translation elongation factor 1-α (*tef1*) genes were amplified and sequenced as described in Woudenberg *et al.* (2017). Consensus sequences were assembled from forward and reverse sequences using Bionumerics v. 4.61 (Applied Maths, St-Martens-Latem, Belgium). All sequences generated were deposited in GenBank (Table 1).

Alignments and phylogenetic analyses

Individual sequence alignments of the ITS, *tub2* and *tef1* datasets were generated with MAFFT v. 7.271 (http://mafft.cbrc.jp/alignment/server/index.html) using the L-INS-i method. The best nucleotide substitution models were determined with Findmodel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). For both the single gene sequence alignments and the concatenated alignment, Bayesian and Maximum-likelihood analyses were performed as described in Woudenberg *et al.* (2017). An additional phylogenetic tree was constructed based on the ITS sequences of a broader selection of isolates representing all species recognized by Sandoval-Denis *et al.* (2016b) and in this study, together with ITS sequences from the Chinese herbarium specimens available in GenBank (Table 2). To demonstrate the placement of two species initially classified in *Cephalotrichum* outside the genus, an alignment and phylogenetic tree based on the ITS and LSU sequences of representative strains of the genera *Acaulium*, *Cephalotrichum*, *Kernia* and *Graphium* was assembled based on the sampling of Sandoval-Denis *et al.* (2016a). The resulting trees were printed with TreeView v. 1.6.6 (Page 1996) and, together with the alignments, deposited in TreeBASE (http://www.treebase.org).

Morphology

Cultures were incubated on oatmeal agar (OA, which favours synnema development), malt extract agar (MEA) and dichloran 18 % glycerol agar (DG18) plates (recipes from Samson *et al.* 2010) at 25 °C in the dark. After 14 d, growth rates were measured and colony characters noted. Colony colours were rated following the charts of Rayner (1970). Dried herbarium material was rehydrated in sterile water, which was then replaced by Shear's mounting media for photomicroscopy (Crous *et al.* 2009). Measurements and descriptions of microscopic structures were made from cultures grown on synthetic nutrient agar (SNA, Samson *et al.* 2010) at 25 °C in the dark for 14 d, mounted in 85 % lactic acid. Macroscopic photographs were made with a Nikon SMZ25 stereo microscope equipped with a Nikon DS-Ri2 high-definition colour camera head. Photomicrographs of diagnostic structures were made with a Zeiss Axio Imager A2 microscope equipped with a Nikon DS-Ri2 high-definition colour camera head, using differential interference contrast (DIC) optics and the Nikon software NIS-elements D v. 4.50.

RESULTS

Phylogeny

The concatenated, multi-gene *Cephalotrichum* phylogeny alignment included sequences of 62 strains and was 1 979 bp long, with the partitions being 566 characters for ITS (67 informative or unique), 884 for *tef1* (103) and 529 for *tub2* (227). The TrN model